recipients of 4-6/6 HLA match grafts when at least 1.7 × 10^5 $CD34^+$ cells/kg are infused. This association was based on the $CD34^+$ counts assessed on thawed products. But the question to be addressed is indeed whether we can predict the thawed $CD34^+$ cell dose based on an analysis of the pre-freeze $CD34^+$ enumeration and the reliability of a $CD34^+$ assessment using an associated aliquot. Therefore, we evaluated fifty duplicate cryopreserved CB aliquots (1.5 ml cryovials) from CB units stored according to the standard banking procedures at the Madrid CBB (MCBB). After shipment using a transport liquid nitrogen container, the aliquots were thawed and processed by the same protocol at both the UM and MCBB. Total pre-freezing NC/ml was 8.21 ± 3.36 × 10^6. Post-thaw counts at UM (6.77 ± 2.92 × 10^6) and Madrid (6.49 ± 3.0 × 10^6) indicated equivalent NC recoveries (83 ± 13% and 79 ± 15%, respectively). Cell viability by trypan blue before freezing was 90 ± 8%. However, there was a large difference between the post-thaw viabilities at the UM (52 ± 10%) and MCBB (81 ± 11%), indicating this technique is far from normalization. Similarly, different CFU-GM scoring criteria at the UM and MCBB (counting all CFU-GM colonies made up of greater than 40 or 20 cells, respectively) gave a lower CFU-GM number at UM *vs.* MCBB (7.6 ± 5.6 *vs.* 20.3 ± 13.6/50000 cells plated). Despite this variable readout, a significant correlation between these values (r = 0.7) was probably related to the consistency of the method and the same culture media used in this study. The $CD34^+$ frequencies (analyzed according to the ISHAGE dual-platform protocol) were also discrepant. While the post-thaw %CD34 was 0.98 ± 0.66 at the UM, it was 0.57 ± 0.39 at the MCBB (with the pre-freeze %CD34 being 0.35 ± 0.22). Bland-Altman and Intraclass Correlation tests displayed considerable lack of agreement and no consistent bias was observed between post-thaw $CD34^+$ at UM and either pre-freeze or post-thaw $CD34^+$ values at MCBB. Thus, the $CD34^+$ cell analysis at the UM and MCBB could not be used interchangeably. However, linear regression showed a significant relationship ($p < 0.001$). The linear equations estimated for each prediction model were: i) y = 2.758x + 0.03121 (post-thaw [pt] UM from pre-freeze [pf] MCBB counts); ii) y = 1.534x + 0.111 (pt UM from pt MCBB counts); and iii) y = 1.684x + 0.00567 (pt MCBB from pf MCBB counts). The coefficient of determination (R^2_a) for each model was 0.85, 0.82 and 0.80 respectively. Therefore, as high as 85% and 82% of the total variance of post-thaw $CD34^+$ values at the UM was explained respectively by the variation in pre-freeze (first model) or post-thaw (second model) CD34 values at the MCBB. Importantly, all but one of the observed $CD34^+$ counts were above the lower 90% individual prediction limits. In summary, regardless the variability of $CD34^+$ cell enumeration at the UM and MCBB, it is possible to foresee with a confidence of 95% the $CD34^+$ cell dose that would be infused to CB transplantation patients at the UM, either from the pre-freeze MCBB data or from the $CD34^+$ assessment using a cryopreserved aliquot. Whereas this regression model is only valid between the UM and MCBB, this approach could be widely applied to predict the transplantation outcomes from $CD34^+$ data provided by all CB banks. Finally, on examination of the flow cytometry data files, the CD34 enumeration discrepancy was seemingly due to differences in the initial cell acquisition performed at the UM and MCBB. This discrepancy could be minimized by adopting uniform operating procedures.

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STANDARDIZATION OF CFU ASSAYS

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The advantages of using cord blood derived cells for the treatment of leukemia and other hematological malignancies are now well recognized. A recent regulatory ruling has mandated that a functional test (e.g. a colony forming cell (CFC) assay) evaluating the proliferative potential of the cells within the product following cell processing and freezing must be performed if the sample is to be used for transplantation. This highlights the need for a standardized assay. Previously published data have reported significant variability in the quantification of the hematopoietic progenitors using the CFC assay. Originally, recognition and enumeration of the colonies was believed to account for this variability, however variation in CFC quantification could also be caused by different media formulations or technical errors in sample preparation, cell counting and dilution. Two distinct proficiency testing programs were designed to assess the contribution of these various parameters to the variability. The preliminary program, with 54 participants within North America, sought to determine the variability in the recognition and enumeration of CFC. This was achieved by determining the coefficient of variation (CV) for CFU-GM and total CFC enumeration when sample preparation events were controlled. The second program was global, and also assessed the contribution of the cell preparation steps to the overall variability of the CFC assay. Participants (n = 134) were provided with identical vials of frozen bone marrow cells and were instructed to thaw, wash, count, assess viability and dilute the cells prior to their addition to a standardized formulation of MethoCult™. Fourteen days later, participants quantified myeloid and erythroid colonies as in the original tests. The considerable increase in the CVs in this second test confirmed that sample preparation steps contribute significantly to the variability in this assay. Standardized protocols for cell preparation and training will decrease this variability and facilitate global applicability of data generated from various laboratories.

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UNRELATED CORD BLOOD TRANSPLANTATION IN PEDIATRIC PATIENTS WITH NON-MALIGNANT DISEASES

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The COBLT or Cord Blood Transplantation Study was a multi-institutional study sponsored by the National Heart Lung and Blood Institute. It was comprised of a banking and transplantation study. The banking study established 3 banks which employed common standards for donor screening and recruitment and cord blood collection, processing, testing, cryopreservation and storage. Screening of 34,700 women yielded 24,200 eligible for the study who were approached for consent to participate. Eighty-five percent (20,710 women) were consented and 17,207 were collected, 47% of which were discarded for low cell count, infectious disease or maternal history exclusion or problems with processing. Of note, a lower percentage of African American donations were eligible for banking because they tended to have lower cell counts per unit volume. Approximately 9112 units were moved to long term storage and available for transplantation. The transplantation study employed common protocols for preparative regimens, GvHD prophylaxis and supportive care. Uniform definitions of engraftment, GvHD and toxicity scoring, graft failure, causes of death and relapse were employed. Strata were developed for children and adults with malignant and non-malignant conditions. This report focuses on outcomes in 69 patients with inborn errors of metabolism transplanted between August of 1999 and June of 2004. The median age of the patients was 1.8 years (range 0.1-11.7 years). There was a predominance of males (55%) and Caucasians (74%). The patients were diagnosed with MPS syndromes (57%), ALD (12%), MLD (6%), Krabbe Disease (23%) and Tay Sachs Disease (4%). Only 25% of patients were CMV seropositive before transplant. A significant portion of the patients (35%) had poor performance status defined by a Lansky score ≤80%. The COBLT banks provided 70% of the donor units used in the study. The median total nucleated cell dose and CD34 cell dose selected were 8.7 × 10^7/kg and 2.4 × 10^5/kg, respectively. HLA matching was performed using molecular typing at an intermediate resolution for HLA Class I A and B and high resolution for DRB1. High resolution matching was scored retrospectively. The majority of patients received grafts mismatched at 1 (45%) or 2 (48%) HLA loci. After high resolution typing, 23% of patient/donor pairs were demoted to lesser matches. Patients were prepared for transplant with busulfan (16 doses with first dose pharmacokinetics targeting a steady state of 600-900 ng/ml), cyclophosphamide 200 mg/kg and equine ATG 90 mg/kg. GvHD prophylaxis was delivered with methylprednisolone and cyclosporine. The cumulative incidence